In the course of differentiation, in the overwhelming majority of cases, the cell genotype remains unchanged. However, tissue differences are inherited during cell reproduction (histiotypic growth). This means that the effects of determining influences are as a rule totally or almost totally irreversible, and hence only de-differentiation and not de-determination can take place. There is no return to a state from which different ways of development may be chosen.

Nuclear changes leading to stable differences in the rate of synthesis of many individual proteins between tissues (which we shall call epigenomic changes) have been little studied. Although these changes might reflect an inherited pattern of gene repression, neither the mechanism of gene repression in somatic cells nor that of the maintenance of repressed and de-repressed states during multiplication are known.

One of the main obstacles to the study of epigenomic variability is the lack of methods for defining simple tissue differences in the synthesis of individual proteins. Embryonic induction is not identical with the simplest reactions of gene repression and de-repression in bacteria since the immediate effect of embryonic inducers is only one link in an extended process involving many aspects of cellular activity.

A possible approach is offered by the antigenic structure of tumour cells, particularly in cases where the levels of some antigens in the tumour are higher than in the normal homologous tissue. Weiler (1952) has studied a hepatoma, the cells of which contained antigens produced in the kidney and spleen, but not in the homologous normal organ, the liver. A similar case has been studied in detail by Day (1965). For his experiments he used a labelled antihepatoma serum. Injecting it into rats he proved that antibodies were localized in the hepatoma and kidney but not in the liver.

We have undertaken a detailed study of this phenomenon on three groups of

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histogenetically different tumours induced in rats. The results obtained are
given below. Antigens found in the tumour cells that are proper to tissues other
than the tissue of origin of the tumour we have termed ‘hetero-organic antigens’.

MATERIALS AND METHODS

A total of fifty-five rat tumours induced in our laboratory and maintained by
transplantation were used. Sixteen of them were myogenic, twenty-seven were
hepatocellular and twelve were kidney tumours.
The myogenic tumours belonged to ten lines, each obtained by transplantation
of a tumour developed after the implantation of newborn rat cells made
malignant in monolayer cultures into a Wistar rat. Malignancy was noted after
12 months cultivation in two subcultures irradiated with 100 r and 100 r x 5
immediately after explantation and also in a non-irradiated subculture (Vakhtin,
Ignatova, Surikov & Tsikarishvili, 1963a, b). From histological examination
most of the myogenic tumours were regarded as rhabdomyosarcomas and
rhabdomyoblastomas (Shvemberger & Vakhtin, 1965).

Hepatocellular tumours were induced in outbred rats by repeated peroral
injection of an aqueous solution of N-diethylnitrosamine. Each dose of car-
cinogen was 0.5–1.5 mg per rat, the total dose ranging from 40 to 70 mg.
Tumours appeared in livers on the average at 40 weeks after the treatment
started (Shvemberger, 1963). In some cases the hepatomas induced in this way
were transplantable. The tumours used for the present investigation were
taken from two transplantable lines (N 15 and N 27).

Kidney tumours were induced in outbred rats with N-dimethylnitrosamine.
For this purpose an aqueous solution of the carcinogen was injected into rats
through a stomach tube for 5 days. Each dose was 2.0 mg, the total dose 10.0 mg.
As a consequence of this treatment kidney tumours were developed (dif-
ferentiated epithelial adenocarcinomas and anaplastic tumours). The average time
of tumour induction was about 60 weeks (Shvemberger, 1965). Transplantable
tumours were taken from a stock tumour line obtained as a result of successful
transplantation of a primary tumour, adenocarcinoma 12.

Saline extracts of tumour were used as antigens for testing. After the removal
of all necrotic areas, blood clots and connective tissue the tumours were
ground in a homogenizer with two volumes of 0.14 M-NaCl solution. The homo-
genate obtained was centrifuged for 30 min (12000–15000 rev/min), the sedi-
ment was discarded, and the supernatant left overnight at 4 °C. If a precipitate
formed it was removed by recentrifugation. The antigen solutions were pre-
served with merthiolate (final concentration 10⁻⁴). The protein content was
measured by the biuret test. The antigen-containing preparations were then
diluted with 0.14 M-NaCl solution to give equal protein contents. Saline extracts
of different normal tissues from intact rats were prepared in the same way.

Organ-specific (anti-muscle, anti-liver and anti-kidney) immune sera and
Antigenicity of tumour cells

immune sera against myogenic tumours of transplantable line 3/1, hepatocellular tumours of line 15 and kidney adenocarcinoma of line 12 were used for these experiments. In order to obtain immune sera rabbits were injected with doses of homogenates equivalent to 2.0–2.5 g of fresh tissue. The immunization procedure was as follows: the first injection was subcutaneous with Freund’s adjuvant; the second was intramuscular after 14 days, and the third also intramuscular, after 7 more days. Every 6–8 weeks the rabbits were further injected intramuscularly with the homogenate for re-immunization.

Organospecific immune sera were prepared from native anti-organ immune sera, the latter being absorbed with extracts from heterologous organs and serum from intact rats. Anti-tumour immune sera were absorbed only against serum antigens of intact rats.

The gamma-globulin fractions were extracted from absorbed immune sera by ammonium sulphate precipitation. The sera (concentrated 3–4 times) were preserved with merthiolate and after proving the completeness of absorption were used without dilution.

The antigenic structure of the tumours was studied by precipitation reactions in agar gel and by specific inhibition of precipitation (Bjözclund, 1952) using the object-slide micro-modification.

Table 1. Precipitation reaction of water–saline extracts from hepatomas with organospecific anti-kidney and anti-muscle sera.

<table>
<thead>
<tr>
<th>Extract (5 mg/ml)</th>
<th>Organo-specific sera</th>
<th>1</th>
<th>4</th>
<th>9</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
<th>16</th>
<th>17</th>
<th>18</th>
<th>19</th>
<th>23</th>
<th>15¹</th>
<th>15²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-kidney</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-muscle</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In this and following tables the number of (+) equals the number of precipitation bands formed; hepatomas 15¹–15² are the 1st–11th generations of primary hepatoma 15, and similarly for hepatoma 27.

RESULTS

Two methods may be used for the investigation of the hetero-organic antigens of tumour cells—a direct method using organospecific sera and an indirect one using the respective anti-tumour sera. We used both.

The results obtained with organospecific sera will be dealt with first. We
succeeded not only in proving the existence of tumour-cell hetero-organic antigens, but in obtaining some conclusions on their nature (Tables 1–3). In the extracts of some hepatocellular tumours organospecific antigens of kidney and muscle tissue have been discovered, whereas in the extracts of kidney adenocarcinoma only muscle tissue antigens were found. The testing of myogenic tumour extracts with organospecific anti-kidney and anti-liver sera has revealed no organospecific antigens. As one might expect, not a single case of positive reaction was noted in the controls between the organospecific serum and non-homologous normal tissue extract.

Table 2. Precipitation reaction of water–saline extracts from transplantable myogenic tumours with organospecific anti-liver and anti-kidney sera

<table>
<thead>
<tr>
<th>Organo-specific sera</th>
<th>Extract (5 mg/ml)</th>
<th>Organs of intact rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of lines of myogenic tumours</td>
<td>Liver</td>
</tr>
<tr>
<td></td>
<td>1/2 3/1 3/2 3/3 5 5/1 6 8/2 8/3 9/1</td>
<td></td>
</tr>
<tr>
<td>Anti-liver</td>
<td>— — — — — — — — — —</td>
<td>+ + +</td>
</tr>
<tr>
<td>Anti-kidney</td>
<td>— — — ± — — — — — —</td>
<td>—</td>
</tr>
</tbody>
</table>

Table 3. Precipitation reaction of water–saline extracts of kidney tumours with organospecific anti-liver and anti-muscle sera

<table>
<thead>
<tr>
<th>Organo-specific sera</th>
<th>Primary tumours</th>
<th>Second passage</th>
<th>Extracts (7-5 mg/ml)</th>
<th>Third passage</th>
<th>Fourth passage</th>
<th>Organs of intact rats</th>
<th>Skeletal muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-liver</td>
<td>34 35 36 12a 12b 12a 12b 12a 12a 12a 12a 12a 12a 12a 12b 12b</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>+ +</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Anti-muscle</td>
<td>— — — — — — — — — ± — — — + —</td>
<td>—</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In the indirect method using anti-tumour sera indications of the presence in tumour cells of some antigens characteristic of organs not homologous to the tumours were obtained. The extract of normal tissue homologous to the tumour was placed in a well in the agar plate in a concentration sufficient for total neutralization of antibodies to the antigens of normal tissue. The anti-tumour serum is later placed in the same well and has to pass a kind of filter formed by the extract of normal tumour-homologous tissue diffused in the agar gel. This serum is then tested with extracts of normal tissues not homologous to the tumour.

One of the experiments of this series is shown in Table 4. After having lost the ability to react with an extract from normal liver the absorbed anti-hepatoma serum reacted not only with the hepatoma extract (two precipitation bands) but
Antigenicity of tumour cells

also with the extracts of normal organs not homologous to the tumour (1–2 precipitation bands). Thus the content of hetero-organic antigens in the extract of normal liver was evidently insufficient to neutralize specific antibodies in the antihepatoma serum. The high content of antibodies against hetero-organic antigens is an indication of the increased content of the latter in the tumour tissue used for the immunization of rabbits.

Table 4. *Precipitation reaction of anti-hepatomic and anti-liver sera with water–saline extracts of organs of intact rats*

<table>
<thead>
<tr>
<th>Sera</th>
<th>Extract</th>
<th>Dose (mg/ml)</th>
<th>Liver</th>
<th>Kidney</th>
<th>Spleen</th>
<th>Lung</th>
<th>Skeletal muscle</th>
<th>Hepatoma 15</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-hepatomic</td>
<td>Rat liver</td>
<td>7.5</td>
<td>–</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15.0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Anti-hepatomic</td>
<td>Hepatoma 15</td>
<td>7.5</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15.0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Anti-liver</td>
<td>Rat liver</td>
<td>7.5</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15.0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

The results obtained could be tentatively explained by an increased immunogenic activity of the hetero-organic antigens. Control experiments persuaded us that such a supposition is not correct; anti-liver serum absorbed with extract of liver tissue in conditions analogous to the experiments with the anti-hepatoma sera was found to be exhausted also in respect of other normal tissue extracts. Similar results were obtained when anti-hepatoma serum was absorbed by its ‘own’ tumour extract. The increased content of hetero-organic antigens in the hepatoma extracts as compared with extracts from a normal liver also cannot be explained as a more efficient extraction from tumours since tumour and normal tissue homogenates have been used for the immunization of rabbits and not extracts.

These data show that hetero-organic antigens of tumour cells have nothing in common with antigens of the Forssman type or with isoantigens (1). In some cases hetero-organic antigens proved to be identical to organospecific antigens of organs not homologous to the tumour under investigation. (2). In the inverse experiments anti-tumour sera after absorption with extracts from normal tissues homologous to the tumours never failed to react with the extracts of non-homologous tissues. It cannot be thought that the reaction depended upon antigens of the Forssman type since in that case the amount of antigen in the organs homologous to tumours would always have been lower than in heterologous organs.

It is remarkable that while the absorption of anti-hepatoma serum with liver
extracts at higher concentrations led to a decrease in the number of positive reactions with the extracts of normal tissues heterologous to the hepatoma, in the case of positive reactions the number of detectable hetero-organic antigens was reduced. The quantitative character of the differences in the hetero-organic antigen content in tumour and normal tissue applies also to rhabdomyosarcoma and kidney adenocarcinoma.

The quantitative characteristics of this phenomenon were also investigated with organospecific sera. Table 5 describes a relevant experiment. Evidently the organospecific anti-muscle serum can detect one organospecific antigen in the extract of adult rat muscle used for testing at a concentration of 0.5 mg/ml. In the experiments with a transplantable kidney adenocarcinoma similar results could be obtained only by testing an extract at a concentration not less than 3.75 mg/ml, i.e. 7.5 times more than that of muscle. Naturally in the extracts of kidney tissue of adult and newborn rats no organo-specific antigens of muscle tissue has been found.

Table 5. Precipitation reaction of organospecific anti-muscle serum with extracts from muscle, kidney and kidney adenocarcinoma of rats

<table>
<thead>
<tr>
<th>Extract from</th>
<th>Dose of Extract (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15.0</td>
</tr>
<tr>
<td>Adult rat muscle</td>
<td>+ + + +</td>
</tr>
<tr>
<td>Rat kidney adenocarcinoma 124</td>
<td>+ + + +</td>
</tr>
<tr>
<td>Adult rat kidney</td>
<td>-</td>
</tr>
<tr>
<td>Newborn-rat kidney</td>
<td>-</td>
</tr>
</tbody>
</table>

The problem of the identity of hetero-organic antigens in tumours of the same and of different tissue origin is difficult using anti-tumour sera. We limited ourselves to the investigation of organospecific antigens.

Using anti-kidney organospecific serum we found one organospecific kidney antigen in each of 15 extracts of hepato-cellular tumours out of 27 extracts studied (including primary and transplantaable hepatomas). Ten of 15 such extracts (7 of primary and 3 of transplantable hepatomas) were tested for antigenic identity in different combinations on one slide (Table 6). Not all the possible combinations have been studied (23 out of 45), but in all cases when a comparison was made we obtained a positive result indicating that the same kidney organospecific antigen is synthesized by the cells of hepatocellular tumours.

Using anti-muscle organospecific serum, the extracts of two transplantable kidney tumours (of twelve investigated) contained organospecific muscle antigens—one antigen in tumour 124 and two in tumour 123. Organospecific muscle antigens were detected also in two primary hepatomas N 16 and N 17. Owing to this we had an opportunity to compare tumours differing in their
Antigenicity of tumour cells

It was found out that hepatoma 16 and kidney adenocarcinoma 12* contained the same organospecific muscle antigen. Another common antigen was revealed in hepatoma N 17 and adenocarcinoma 12*. Hence an antigenic reconstruction of the cell in the conversion to malignancy may have some features in common not only in tumours of the same tissue of origin but also for tumours of different origin.

Table 6. Identity of hetero-organic antigens of hepatocellular tumours of rats studied with the help of organospecific anti-kidney serum

<table>
<thead>
<tr>
<th>No. of hepatomas compared</th>
<th>No. of hepatomas compared</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>.</td>
</tr>
<tr>
<td>12</td>
<td>.</td>
</tr>
<tr>
<td>13</td>
<td>.</td>
</tr>
<tr>
<td>15</td>
<td>.</td>
</tr>
<tr>
<td>16</td>
<td>.</td>
</tr>
<tr>
<td>19</td>
<td>.</td>
</tr>
</tbody>
</table>

Note: + indicates antigenic identity.

DISCUSSION

Our data suggest that several tumours of different histogenesis have an increased content (as compared with homologous normal tissue) of some antigens detectable in one or more organs not homologous to the tumour in question.

These results can be explained satisfactorily only by assuming de-repression of the genes controlling synthesis of the proteins tested. Their presence cannot be due to the mutation of structural genes since the antigens in question are a part of the ‘set’ of a normal genome. The assumption that mutations selectively involving gene regulators occur in tumour cells would be quite arbitrary. Hence there is every reason to consider these changes in the antigenic structure of tumour cells as identical or similar in their nature to those epigenomic changes which are accumulated in the process of cell differentiation.

A similar conclusion may be drawn from evidence of the synthesis of embryonic antigens or their components by the cells of some tumours (Abelev et al. 1963; Potter, 1964; Fel et al. 1966a, b, c).

It seems natural to suggest that as well as a de-repression of some genes there is also a repression of the activity of genes controlling the synthesis of organospecific proteins in tumour cells. This would explain the frequent occurrence of antigenic simplification in tumours; that is, the absence or reduction in content of a number of antigens characterizing homologous normal specialized cells.
However in this case, unlike the case of de-repression, one cannot exclude another explanation in which this phenomenon is determined by mutations.

The existence in tumour cells of hormones which are not elaborated in detectable quantity in homologous normal cells is another example demonstrating de-repression of synthetic processes. Such ectopic hormones are not rare phenomena in tumours. In some lung and liver tumours and even in those of the peripheral nervous system adrenocortical and parathyreohormones have been found. The amount of ectopic hormone produced by tumour cells may be so high as to affect the hormonal status of the bearer of the tumour (Liddle, Givens, Nicholson & Island, 1965; Lipsett, 1965; Munson, Tashjian & Levine, 1965).

As judged by the evidence of bacterial genetics, repressed genes are not completely inactivated (see e.g. Jones & Gollant, 1964). The same has been established for repressed loci controlling the antigenic properties of Paramecium (Seed, Shafer, Finger & Heller, 1964). There are similar data on somatic cells. The quantitative determinations of collagen in cells of various tissues have shown its presence in cells of non-connective tissue origin, though in minor amounts. In epithelial cells, for example, the amount of collagen was found to be thirty times less than in cultures of fibroblasts after their period of intensive reproduction (Green & Goldberg, 1965). These data invite a preliminary conclusion about the range of differences determined by the repressed and de-repressed state of genes in somatic cells. There are similar data on hormone production outside endocrine glands. Small amounts of hormones are produced by cells of many tissues (Tait, 1965). Such data indicate the necessity of defining the term 'organospecific antigens'. It is quite possible that small amounts of these antigens are synthesized in tissues of an adult organism other than the tissues for which they are considered as specific.

De-repression which we observed in some of the tumours was never complete. The example given in Table 5 shows a difference of 7–8 times between the tumour and the organ for which the given antigen is specific. It is not known whether such differences are inevitable.

A more definite conclusion can be drawn about another fact revealed in the identity tests. There is a clear tendency for certain genes to be preferentially involved in the process of de-repression in different tumours of the same tissue origin (Table 6). This tendency is seen even when comparing tumours of different histogenesis. This means that epigenomic changes determining intertissue differences in the synthesis of individual proteins differ in their stability.

All the available data indicate that the de-repression of genes repressed in the original normal tissue is not a rare phenomenon in tumour cells. Of course the results obtained are limited by the sensitivity of the method used. Precipitation in agar reveals only those proteins in which the tested extract is rich. These will be a small minority of all cell proteins. It is likely that some tumours contain many other undetectable proteins which are also controlled by de-repressed genes and
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which are identical with specific proteins of tissues which are not homologous
to the tumour. Hence tumours may be used to distinguish elementary tissue
differences involving the synthesis of individual proteins.

At the same time the morphology of tumours of all the three groups studied
(hepatomas, myogenic tumours, kidney tumours) betrays their histogenesis.
Hence, elementary intertissue differences, although probably interrelated, do not
constitute a system where a change of individual components inevitably
involves alterations of the remaining parts of the system. The usual statements
on the irreversibility of determination should be considered in the light of these
findings.

The results of morphological investigations suggest the complete stability of
tissue differences, or more accurately the major visible differences that exist for
example between connective tissue and epithelia. Many investigators made
attempts to find exceptions to this rule (see, for example Reuer, 1954; Trinkaus,
1956; Petrakis, Davis & Licia, 1960), but usually with negative results even for
tumour material. No doubt, in any case, such exceptions are extremely rare.

However, morphological evidence reflects only the remote result of many
elementary processes. Intertissue differences are based on the differences of these
processes arising in epigenomic way. There is no reason to assume that the
elementary processes leading to the final result are also in all cases individually
irreversible. The presence in tumour cells of antigens characteristic of tissues
non-homologous to the tumour confirm the validity of such an interpretation.

SUMMARY

In a number of tumours differing in their tissue of origin the amount of
certain antigens usually found in organs not homologous with the tumours in
question (hetero-organic antigens) appear to be increased as compared with the
normal homologous tissue. These data are interpreted upon the assumption
that in the tumour cells there occurred a de-repression of genes controlling the
synthesis of the proteins tested. The results of identification of hetero-organic
antigens and their quantitative characteristics are presented.

The data suggest that epigenomic elementary intertissue differences involving
the synthesis of individual proteins, although perhaps being interrelated, do not
constitute a system in which the change of some components inevitably results
in alterations of remaining elements. The significance of these data in relation to
the irreversibility of determination is discussed.
РЕЗЮМЕ

Нарушение антигенной структуры у опухолевых клеток, как материал для изучения тканевой дифференциации

В нескольких опухолях крыс, относящихся к разным группам по своему гистогенезу, обнаружено повышенное, по сравнению с нормальной гомологичной тканью, содержание некоторых антигенов, присущих органам, негомологичным исследуемой опухоли (гетероорганные антигены). Эти данные можно удовлетворительно объяснить только приняв, что в опухолевых клетках произошла дерепрессия генов, контролирующих синтез тестируемых белков. Представлены сведения о результатах идентификации гетероорганных антигенов и дана их количественная характеристика.

Полученные данные свидетельствуют о том, что возникшие эпигеномным путем элементарные межтканевые различия в синтезе индивидуальных белков, хотя, может быть, и взаимосвязаны, но не представляют собой системы, в которой изменение отдельных компонентов неизбежно влечет изменения остальных частей системы. Рассмотрено значение этих изменений для анализа проблемы необратимости детерминации.

REFERENCES


Antigenicity of tumour cells


(Manuscript received 3 May 1967, revised 16 November 1967)